Canadä

Contractor's Final Report

DSS Contract: File no. 15SC.01K26-6-0167
Serial no. 01K26-6-0167/01SC

FEASIBILITY OF USING ECTOMYCORRHIZAL FUNGI TO ALLEVIATE DROUGHT-RELATED PROBLEMS OF CONTAINERIZED BLACK SPRUCE

ENFOR PROJECT P-338

1988

Contractor:

D. Boyle
Nova Scotia Research Foundation Corp.
P.O. Box 790
Dartmouth, Nova Scotia
B2Y 3Z7

Scientific Authority:

P.O. Salonius
Forestry Canada
Maritimes
P.O. Box 4000
Fredericton, N.B.
E3B 5P7

This work was supported by the Federal Panel on Energy R&D (PERD)

This Report has been reviewed by Forestry Canada and approved for distribution. Approval does not necessarily signify that the contents reflect the views and policies of Forestry Canada.

FOREWORD

ENFOR is the acronym for the ENergy from the FORest (ÉNergie de la FORet) program of Forestry Canada. This program of research and development is aimed at securing the knowledge and technical competence to facilitate in the medium to long term a greatly increased contribution from forest biomass to our nation's primary energy production. It is part of the federal government's efforts to promote the development and use of renewable energy as a means of reducing dependence on petroleum and other non-renewable energy sources.

The ENFOR program is concerned with the assessment and production of forest biomass with potential for energy conversion and deals with such forest-oriented subjects as inventory, harvesting technology, silviculture and environmental impacts. (Biomass Conversion, dealing with the technology of converting biomass to energy or fuels, is the responsibility of the Bioenergy Development Program of the Department of Energy, Mines and Resources). Most ENFOR projects, although developed by Forestry Canada scientists in the light of program objectives, are carried out under contract by forestry consultants and research specialists. Contractors are selected in accordance with science procurement tendering procedures of the Department of Supply and Services. For further information on the ENFOR Biomass Production program, contact ...

ENFOR Secretariat
Forestry Canada
Ottawa, Ontario
K1A 1G5

TABLE OF CONTENTS

	PAGE NO.
INTRODUCTION	1
MATERIALS AND METHODS	4
RESULTS AND DISCUSSION	8
CONCLUSIONS	15
LITERATURE CITED	16
APPENDIX	25

INTRODUCTION

There is good evidence in the literature that mycorrhizal fungi can affect the water status of conifers (Duddridge et al. 1980, Parke et al. 1983, Boyd et al. 1986), but it is not clear which species of fungi would be most effective at improving the performance of drought-stressed trees under field conditions. Direct field testing of large numbers of tree-fungus combinations is not feasible since, as of yet, it is only possible to produce mycorrhizae under nursery conditions with a few fungal species. Furthermore, field site variability and the un-predictability of drought stress for a given season frequently make the results from field testing un-interpretable.

The literature suggests that certain attributes of mycorrhizal fungi or of fungus-tree associations which have been assessed under controlled environment conditions might relate to their ability to confer drought tolerance to seedlings in the field. For example, rhizomorphs or mycelial strands of some fungi have been shown to transport water to the tree seedling (Duddridge et al. 1980, Boyd et al. 1986). If the rhizomorph of a fungus were to penetrate water-containing soils that were not accessible to the tree roots, the mycorrhizae might improve the tree's performance. Certain mycorrhizal species have been shown to grow better under conditions of low water potential (induced using osmotically-active agents such as polythylene glycol) than do other species (Mexal and Reid 1973). It was suggested that these mycorrhizal fungi might enable trees to perform better under

conditions of low water availability than the fungi which could not tolerate low water potential. Some mycorrhizal isolates enable the photosynthetic rate (CO_2 exchange rate) of tree seedlings to recover more rapidly after imposed drought stress under laboratory conditions than do other fungal isolates (Parke et al. 1983). This could possibly indicate better performance on droughty field sites. Some isolates can grow at lower temperatures which might facilitate the colonization of lower soil horizons during the spring when soils were cool, promoting the uptake of water from the lower soil horizons during subsequent drought periods. An ideal mycorrhizal fungus for use on drought-stressed sites might behave favorably when tested for each of these attributes.

The objective of this project was to determine whether controlled environment (laboratory or microplot) screening of candidate mycorrhizal species for attributes suspected to relate to drought tolerance could be used to choose mycorrhizal species adapted for use on drought-stressed field sites. The results of these screening tests are presented in this and in the preceding report (Boyle, 1987).

Our original intention was to determine whether the results obtained in the screening trials correlated with the results obtained with the same isolates on spruce seedlings planted into sites with drought stress. Our field tests have not been entirely successful. First-season field results with H. longicaudum have been presented in the December 1987 ENFOR report. We are still trying to develop methods

that will enable us to obtain high mycorrhizae development with the other isolates under nursery conditions. This work is being conducted under a contract with NSFRAC. Results from these inoculation trials and subsequent field test results, as well as second and third year performance results for H. longicaudum-inoculated seedlings will be presented in reports for that project.

MATERIALS AND METHODS (See also previous report, Boyle, 1987)

Growth rate responses to temperature were calculated from the colony diameter of isolates after 20 days growth on replicate MMN agar plates - held in incubators at the indicated temperatures. Growth rate responses to water potential (Ψ) were calculated from colony diameters of isolates growing on top of glass fiber filter papers placed on top of coarse (#2) silica grit. This was saturated with MMN liquid medium supplemented with the appropriate amount of polyethylene glycol (mw 6000 PEG) for the indicated Ψ. Water potentials of media or soils were determined by dew point depression methods using a Wescor 33 T dew point hydrometer.

Photosynthetic responses of plants were measured using an ADC infrared gas analyzer. Seedling shoots were enclosed in 8 x 25 cm "Zip-Lock" sandwich bags sealed around the plant stem with plasticine (Fig. 2). Air (ca 350 ppm CO_2) was pumped through the bag at 500 ml\min. The difference in the CO_2 content of the influent and effluent streams after a 10 min equilibration period, was used to calculate the apparent photosynthetic rate (APR).

The seedlings were held in a controlled environment room for APR determinations. Under these conditions, APR was light saturated, a 30% drop in light intensity having little effect on the rate of CO_2 uptake. In contrast, air flow rates through the bags did affect the APR, presumably by increasing the air movement within the bag and decreasing

the leaf boundary layer resistance to diffusion of CO_2 into the stomates. Air flow was therefore kept constant throughout the experiments. The bags were removed from the seedlings between measurements, although experiments showed that extensive (e.g. 48h) periods of enclosure did not markedly effect APR.

Seedlings for the experiments in which APR was measured were grown in a controlled environment room using 1:1 peat:vermiculite mix, mycelial slurry mycorrhizal inoculum and fertilization schedules as outlined previously (Boyle et al. 1987). For seedling production, we used a 16 h 22°C 10 klx day and an 8 h 16°C night. The relative humidity was maintained at 80-100%. When the seedlings were 5 months old, dormancy was induced by moving them into an 8 h 2 klx light - 16 h dark photoperiod at 8°C. They were watered 2x/wk. After 2 months the temperature was increased to 20°C. They were then moved back into the long photoperiod conditions, where within about 2 weeks the buds began to flush. The seedlings were then visually assessed for mycorrhizae. Colonized seedlings were used in either the drought simulation experiment (PEG) or in the drought cycle experiments described below.

For the drought simulation experiment (Table 2), the seedlings with their roots and associated mix were heavily watered and their APR was measured. The root plugs were then saturated with 30% PEG (ca -2.4 mPa) and after 3 h the APR was again measured. The roots were then rinsed gently with distilled water (to displace all the PEG) and the APR was measured at intervals.

For the drought cycle experiments (Table 3), colonized seedlings were transplanted into soil-containing systems (Fig. 2). The bags contained a lower mineral soil horizon (ca 15 cm deep) and an upper organic soil horizon (ca 10 cm deep). These soils were collected at the edge of an area behind the NSRFC that had been clear-cut about two years previously. The soils were steamed for 90 minutes about 2 weeks before being placed into the bags. The bags were perforated to facilitate drainage and aeration. All bags received the same weight of soil. After planting they were moved to the controlled environment room, where they were watered twice a week for 2 weeks. Watering was then stopped and the systems were then allowed to dry. They were periodically weighed. After about 1 month, some of the systems were approaching a weight that represented 17% soil moisture. Preliminary experiments and spot checks of tree's APR showed that at this soil moisture, photosynthetic activity was very low. The systems were maintained at this soil moisture by injecting small volumes of water into the root zones using a hypodermic syringe. After about 7 days, all the systems had reached 17% soil moisture. (There was no apparent correlation between the rate of water loss and treatment, the differing rates probably being attributable to minor differences in e.g. the perforation of the bags). The APR of all the systems was then measured and they were then watered up to field capacity with distilled water. At intervals, the APR was again measured.

After the last APR determination, the systems were disassembled and assessed macroscopically for mycorrhizal development (rhizomorphs,

extra-matrical hyphae, root colonization) in the upper (0-10 cm), middle (10-20 cm) and lowest (20-27 cm) parts of the soil column. Root lengths and dry weights were determined as were shoot heights, dry weights and root collar diameters.

RESULTS AND DISCUSSION

In Table 1, we have summarized the main characteristics of the isolates with which we have been able to form mycorrhizae. Detailed presentation of this data is available in the December, 1987 ENFOR project report. It should be emphasized that this data was collected with one strain of each fungal species. Work in various labs is showing that intra-species variation can be at least as great as inter-species variation for many characteristics. Nonetheless, for these strains certain conclusions can be drawn with respect to the likelihood that they would perform well under drought stress. Only isolate 302, Pisolithus tinctorius and Paxillus involutus formed rhizomorphs under any of the conditions investigated (petri plates, growth pouches, multipots or in soil). Of these three fungi, only isolate 302 and P. involutus grew reasonably well at temperatures found in northern latitude soils. Of these two, only isolate 302 could grow at water potentials that are close to those at which tree stress occurs. Based on these considerations, one would predict that this fungus would be the best of those screened for use on Maritime sites with drought stress.

As presented more thoroughly in the previous report (Boyle, 1987), the fungi varied considerably in their growth responses to decreasing water potential. To obtain a clearer understanding as to what these differences might indicate in terms of growth in soil, we collected soil samples at the Delaney Settlement and Knowlan Lake sites and

dried these soils to various water contents (determined gravimetrically). In both soils, the water potential of the mineral soils decreased much more abruptly with decreasing water content than did that of the organic soils (Fig. 1). This indicated that there would be very little difference in the total volume of water available in the mineral layer to fungi which differed in their tolerance to low water potential. The water in a given soil volume would more or less simultaneously become unavailable to both fungi and tree roots, regardless of their ability to metabolize at e.g. -0.1 or -0.2 mPa. The situation was less pronounced in the organic soils, but even here only limited amounts of water are available below -0.1 mPa, which is the approximate Ψ at which trees would be expected to start suffering stress. These results indicate that differences in the fungi's growth rates at various water potentials are not likely to improve tree performance by increasing the amount of water taken up from a given volume of soil. It is more likely that if the fungi are to have an effect on drought tolerance, it will be by exploring deeper soil layers that are otherwise not accessible to the tree.

The apparent photosynthetic rate (APR) of the seedlings with the various mycorrhizae differed both before and after exposure to 30% PEG (Table 2). The differences in APR before exposure to PEG possibly indicated an effect of mycorrhizae on flushing rate, although such an effect was not noticed visually. The seedlings were all approximately the same size. This stimulation was most pronounced with isolate 302 although the other fungi also seemed to have an effect. After 3 h

exposure to the PEG the control spruce seedlings and those with L. laccata underwent a greater decrease in APR than did the seedlings with the other fungi. There was not any correlation between the fungi's effect on the APR and the relative growth rates of the fungi on low PEG-amended MMN agar plates. With the pine seedlings, the fungi did not have any significant effect on the APR response to PEG.

When the PEG was washed off of the seedlings' roots and replaced with water, the APR did not show any marked change until the 4 h readings were taken. By this time, the APR had approximately doubled for most of the treatments compared to what it had been 3 h after PEG exposure. The rate of APR recovery was not markedly altered by fungal treatment. Twenty hours after the PEG was replaced, most treatments were exhibiting slightly more than 50% of their original APR. The incomplete recovery of APR may have been due to toxic effects of the PEG on the seedlings.

These results suggest that some of the fungi (isolate 302, H. longicaudum and to a lesser degree P. tinctorius) helped the spruce seedlings to retain moderate photosynthetic rates in a low Ψ medium. They may have done this by increasing water uptake, decreasing water loss, or in some other manner altering the trees' water status. The fungi did not however appear able to affect the rate at which the trees recovered from water stress when water became readily available. This lack of difference in recovery rate between the treatments might be attributable to the liquid medium, there being no difference in the

volume of medium to which the roots and fungi had access.

When seedlings with the same fungi were grown in soil-containing systems and subjected to a drought cycle, the APR of all fungal treatments within a tree species was approximately the same when the water content of the soil was limiting (Table 3). For most of the spruce treatments this was close to 0, while for the pines it was slightly higher. The trees were quite large at the time of assay (about 4 wks after transplanting into the systems). See Table 4 for yield data. The APR of non-water stressed trees of this size was at least 10x higher.

When the seedlings were re-watered, the APR of some fungal treatments on the spruce trees responded to a much greater extent than did that of others. Control seedlings and those inoculated with L. laccata and P. tinctorius did not regain high photosynthetic rates after either 24 or 125 hours. Many of the control seedlings and those colonized by L. laccata showed signs of permanent wilting (incipient necrosis would be more accurate). In contrast, the APR of the trees inoculated with the other fungi increased dramatically.

The fungi did not have as pronounced an effect on the APR of the pines. There were some differences in the APR of the treatments at 5 h after watering, but these differences did not seem to correlate clearly with fungal treatment. The APR 24 h or 125 h after watering was highest for the pine seedlings inoculated with isolate 302, and for the

control seedlings.

To determine the underlying causes of the effects that the mycorrhizal fungi had on the trees' performance during the drought cycle, we looked at the root, shoot and mycorrhizae development of the trees (Table 4). The number of seedlings within each treatment (N) was small, so only large differences in performance could be detected. We have not yet completed the statistical analysis for the spruces, but these can be anticipated by inspecting the standard deviations.

Statistics aside, the fungi do seem to have had an effect on the growth of both the spruces and pines. Since the seedlings were the same size when they were planted into the systems, these differences reflected growth differences which occurred during the drought cycle. Most of the growth parameters of the spruces were increased by the various fungi. A notable exception was the root length of the treatments inoculated with L. laccata. When we were disassembling the systems, we noticed that the roots of the control seedlings and those colonized with L. laccata had not penetrated very far into the soil below the plug. Since most of the available water was in the lowest soil layers at the end of the drought period (the soils in the bottom of the bags were visibly moist while the soil in the top looked dry) the poor root extension could account for the poor recovery of APR when the control and L. laccata-inoculated spruce seedlings were re-watered. The shoot development of the L. laccata-treated seedlings as reflected by shoot height, root collar diameter and shoot weight was

greater than that of the controls, so their water demand would also have been greater. These factors could account for the low APR of the L. laccata-inoculated spruce seedlings during the drought cycle.

With the pines, many of the root systems extended to the bottom of the bags (26 cm) by the end of the drought cycle. The roots were shorter for H. crustuliniforme. It is not clear why this did not adversely affect the photosynthetic rate. There does not seem to be any clear correlation between any of the growth parameters measured and the APR of the pine seedlings. The pines seem to have some physiological mechanism that does not relate to root size or mycorrhizal development that controls their APR.

The fungi showed interesting differences in their growth into the soil in the bags (see the three columns at the right of Table 4). In the spruce treatments, mycorrhizal hyphae only extended into the lowest soil layers with isolate 302. This isolate also made hyphal strands (possibly rhizomorphs) which extended to the bottom of the soil column. It is the only one of the fungal treatments in which the hyphae extended at least as far into the soil as did the roots. This fungus therefore had the potential to access water that might not have been available to the roots. In most of the fungal treatments, extramatrical hyphal development was only moderate and was limited to the area around the original root plug. They did not spread extensively into the soil. Hyphal spread into the soil into which seedlings are planted may well be the main factor controlling whether

they have significant effects on the seedling's performance, regardless of whether the stress is due to lack of nutrients or lack of water.

In the pine treatments, mycorrhizal development was generally less, and vertical extension of the hyphae into the soil was restricted to the upper 20 cm. Hyphal strands were not detected. This poor development might account for the lack of effect that the fungi had on the pine APR's response to drought.

CONCLUSIONS

The results of this project support the contention that mycorrhizal fungi can affect the ability of a tree to withstand drought stress, and they give some indication as to which isolates should be the best candidates for field testing. Isolate 302 generally showed the most promise. The other isolates showed poor performance in at least one screening test. However, edaphic factors associated with specific field sites might have a greater effect on the isolates' performance than the factors considered here. For example, competition with indigenous mycorrhizal fungi and compatibility of the introduced fungi with the soil at the field site might be the most important factors. Before it will be possible to say whether any of the pre-screening tests used here have validity, it will be necessary to conduct field testing. Field testing will be conducted as soon as we can successfully introduce the fungi of interest onto the roots of adequate numbers of seedlings in the nursery.

LITERATURE CITED

Boyd, R., R.T. Furbank, D.J. Read. 1986. Ectomycorrhizae and the water relations of trees. In: Physiological and Genetical Aspects of Mycorrhizae. Edited by V. Gianinazzi-Pearson and S. Gianinazzi, INRA Paris.

Boyle, C.D. 1987. Feasibility of using ectomycorrhizal fungi to alleviate drought-related problems of containerized Black Spruce. Interim Report, ENFOR P-338.

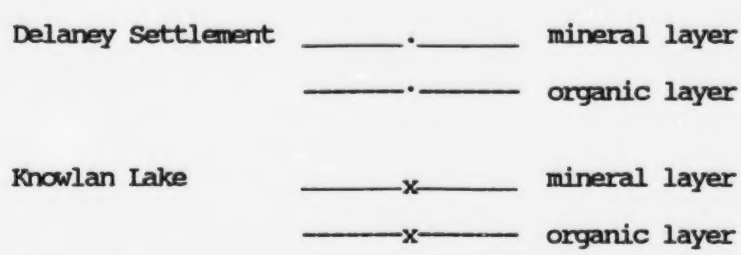
Boyle, C.D., W.J. Robertson and P.O. Salonius. 1987. Use of mycelial slurries of mycorrhizal fungi as inoculum for commercial tree seedling nurseries. Can. J. For. Res. 17: 1480-1486.

Duddridge, J.A., A. Marlibar, D.J. Reid. 1980. Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport. Nature. 287. 834-836.

Mexal, J., C.P.P. Reid. 1973. The growth of selected mycorrhizal fungi in response to induced water stress. Can. J. Bot. 51. 1579-1588.

Parke, J.L., R.G. Linderman and C.H. Black. 1983. The role of mycorrhizae in drought tolerance of Douglas fir seedlings. New Phytol. 95, 83-95.

Figure 1. Water potential of mineral and organic layers of forest soils as a function of water content.



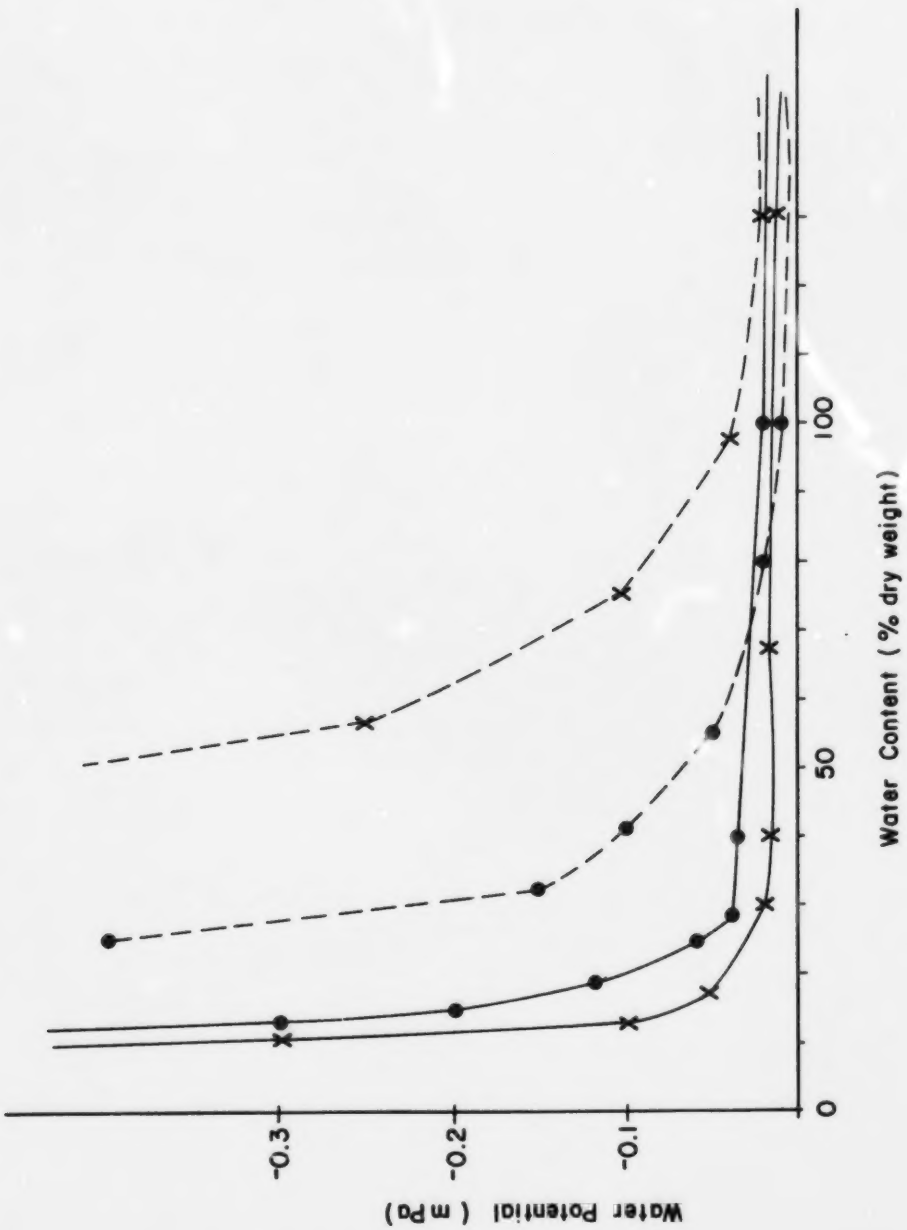

Water Potential (mPa)
-0.3
-0.2
-0.1
0
50
100
Water Content (% dry weight)

Figure 2. System for measuring the photosynthetic response of the plants to drought stress.

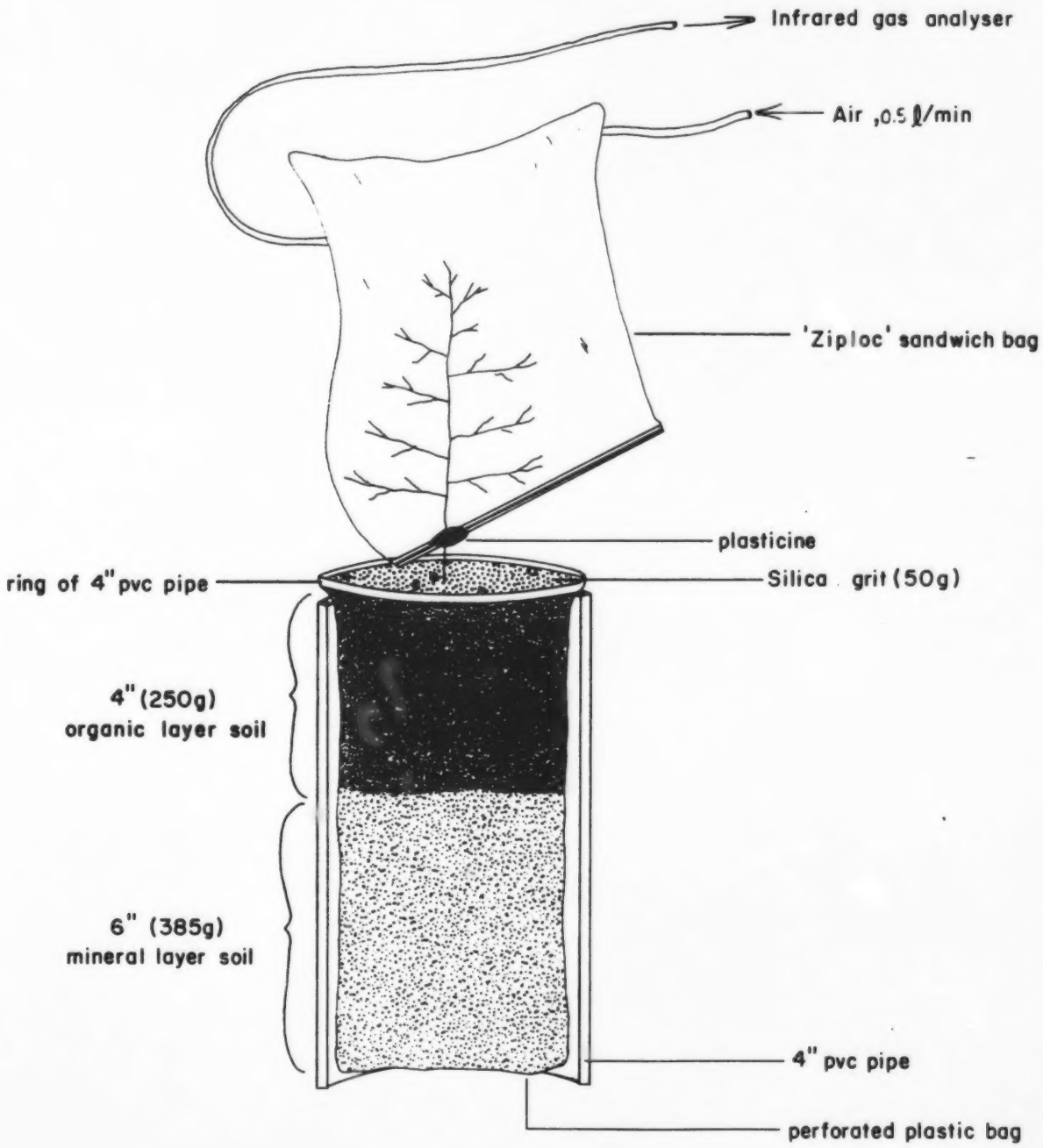

Infrared gas analyser
Air ,0.5 l/min
'Ziploc' sandwich bag
plasticine
ring of 4" pvc pipe
Silica grit (50g)
4" (250g)
organic layer soil
6" (385g)
mineral layer soil
4" pvc pipe
perforated plastic bag

Table 1. Summary of data for isolates of interest in axenix culture (from December 1979 report).

Fungus	Radial Growth Rate (% of Maximal) 12% Peg 8000 ($\Psi \approx -0.1$ mPa)	Radial Growth Rate (% of Maximal) 15°C	Rhizomorphs or Hyphal Strands
Isolate 302	96	38	+
H. longicaudum	35	100	-
H. crustuliniforme	80	44	-
Laccaria laccata	0	66	-
Pisolithus tinctorius	0	3	+
Paxillus involutus	0	78	+
Cenococcum graniforme	0 (variable)	25	-
Thelephora terrestris	25	18	-

Table 2. Apparent photosynthetic rate of seedlings before and after 3h saturation of root plug with 30% PEG (-0.24 mPa), and at various times after re-saturation with water.

Treatment	Apparent Photosynthetic Rate: Initial Before Exposure to PEG ($\mu mol\ CO_2 \cdot h^{-1}$)	After 3h Exposure to PEG (% of Initial Rate)	After PEG Replaced with Water (% of Initial Rate): 1h	4h	20h
Spruce:					
Control	1.2 ± .2[a]	9 ± 8[a]	9 ± 11[a]	33 ± 21[a]	57 ± 47[a]
Isolate 302	2.4 ± .4[b]	25 ± 16[ab]	23 ± 12[a]	52 ± 11[a]	49 ± 9[a]
H. longicaudum	1.6 ± .6[ab]	34 ± 6[b]	27 ± 15[a]	68 ± 19[a]	52 ± 13[a]
L. laccata	2.1 ± .5[b]	10 ± 3[a]	12 ± 2[a]	43 ± 11[a]	49 ± 20[a]
P. tinctorius	1.8 ± .8[ab]	18 ± 17[ab]	17 ± 14[a]	52 ± 5[a]	57 ± 30[a]
Pine:					
Control	1.9 ± .3[a]	21 ± 8[a]	28 ± 13[a]	42 ± 2[a]	61 ± 27[a]
Isolate 302	3.0 ± .4[b]	17 ± 10[a]	22 ± 10[a]	46 ± 10[a]	63 ± 22[a]
H. longicaudum	2.3 ± .2[a]	31 ± 12[a]	27 ± 10[a]	48 ± 9[a]	59 ± 10[a]
L. laccata	2.4 ± .1[a]	12 ± 9[a]	14 ± 16[a]	33 ± 9[a]	58 ± 12[a]

NOTE: Mean ± SD, n = 3. Numbers in columns for a tree species followed by the same letter do not differ significantly at p = 0.05.

Table 3. Apparent photosynthetic rate of tree seedlings with various mycorrhizae in dry soil, and at various times after watering.

	Apparent Photosynthetic Rate (μmol $CO_2 \cdot h^{-1}$)			
	When Dry (410 g)	Hours After Watering		
Spruce		5	24	125
Control	-0.1 ± .0[a]		0.3 ± .8[a]	0.3 ± .6[a]
Isolate 302	0.1 ± .3[a]		1.7 ± .8[b]	1.7 ± 1.0[b]
H. longicaudum	0.4 ± .6[a]		2.4 ± .9[b]	2.0 ± 1.4[b]
L. laccata	0.0 ± .0[a]		0.3 ± .5[a]	.3 ± .6[a]
P. tinctorius	0.1 ± 0[a]		0.9 ± 1.3[ab]	.3 ± 0[ab]
P. involutus	-0.1 ± .2[a]		0.9 ± 1.9[ab]	1.5 ± 2.0[ab]
Pine				
Control	0.4 ± .4[ab]	0.9 ± .8[ab]	1.6 ± 1.3[a]	1.9 ± .8[ab]
Isolate 302	0.6 ± .4[b]	1.9 ± 1.2[ab]	1.8 ± 2.6[a]	3.0 ± .9[ab]
H. longicaudum	0.3 ± .4[ab]	1.0 ± .8[ab]	1.3 ± 1.1[a]	1.5 ± .9[a]
L. laccata	0.4 ± .4[ab]	0.5 ± .1[b]	1.2 ± .5[a]	1.4 ± .3[a]
P. tinctorius	0.9 ± .5[b]	1.1 ± .2[a]	1.6 ± .6[a]	1.8 ± .5[a]
P. involutus	0.1 ± .2[a]	0.4 ± .3[ab]	0.9 ± .4[a]	1.0 ± .3[a]

NOTE: Mean ± s.d. Values in columns for a tree species followed by the same letter do not differ significantly at $p = 0.05$, t = test.

Table 4. Root, shoot and mycorrhizae development of seedlings at end of drought cycle.

	N	Shoot Height (cm)	Root Length (cm)	Root Collar Diameter (mm)	Shoot Dry Weight (gms)	Root Dry Weight (gms)	Mycorrhizae and Rhizomorphs 0-10 cm	11-20 cm	21-26 cm
Spruce:									
Control	5	13.7 ± 0.8	13.4 ± 2.7	2.3 ± 0.2	0.9 ± 0.2	0.4 ± 0.1	-	-	
Isolate 302	5	15.2 ± 0.7	20.4 ± 5.9	2.8 ± 0.2	1.5 ± 0.2	0.7 ± 0.2	+++ R	+++ R	+ R
H. longicaudum	4	16.4 ± 2.7	23.3 ± 2.8	2.7 ± 0.3	1.4 ± 0.1	0.8 ± 0.2	++	+	-
H. crustuliniforme	3	15.2 ± 1.7	18.7 ± 5.3	2.6 ± 0.3	1.6 ± 0.3	0.6 ± 0.1	-	-	-
L. laccata	3	17.0 ± 1.6	11.3 ± 1.9	2.8 ± 0.3	1.1 ± 0.5	0.6 ± 0.1	++	-	-
P. tinctorius	2	15.0 ± 1.0	16.0 ± 1.0	2.6 ± 0.0	1.3 ± 0.1	0.8 ± 0.2	++ R	+	-
P. involutus	3	16.0 ± 2.3	18.0 ± 6.6	2.5 ± 0.3	1.4 ± 0.3	0.6 ± 0.3	-	-	-
Pine:									
Control	5	8.4 ± 1.2^{a}	$>26.0 \pm 0.0^{a}$	2.6 ± 0.2^{a}	2.1 ± 0.4^{a}	1.2 ± 0.1^{a}	-	-	-
Isolate 302	5	10.6 ± 2.3^{b}	$>26.0 \pm 0.0^{a}$	3.2 ± 0.7^{a}	2.0 ± 0.7^{a}	1.1 ± 0.4^{a}	+++	+	-
H. longicaudum	5	7.6 ± 1.2^{a}	$>26.0 \pm 0.0^{a}$	2.5 ± 0.1^{a}	1.2 ± 0.2^{b}	0.6 ± 0.1^{bc}	+++	+	-
H. crustuliniforme	3	7.2 ± 0.6^{a}	13.0 ± 3.0^{c}	2.3 ± 0.4^{a}	1.0 ± 0.2^{b}	0.7 ± 0.1^{bc}	-	-	-
L. laccata	3	7.5 ± 0.4^{a}	24.7 ± 1.9^{a}	2.7 ± 0.5^{a}	1.0 ± 0.1^{b}	0.4 ± 0.0^{b}	+	-	-
P. tinctorius	4	7.4 ± 0.7^{a}	$>26.0 \pm 0.0^{a}$	2.7 ± 0.2^{a}	1.2 ± 0.1^{b}	0.6 ± 0.1^{bc}	-	-	-
P. involutus	4	7.9 ± 0.6^{a}	21.5 ± 4.5^{b}	2.8 ± 0.3^{a}	1.2 ± 0.3^{b}	0.7 ± 0.1^{c}	-	-	-

NOTE: Mean ± sd. Values in columns for a tree species followed by the same letter do not differ significantly at $p = 0.05$, 1 sd test. +++, ++, + and - = abundant, moderate, slight or no mycorrhizal hyphae extending into soil. R = rhizomorphs or hyphal strands present.

APPENDIX

There were a number of experiments that we carried out during this contract which did not succeed. I will mention these here, just for the record.

The experiment in which seedlings with the various mycorrhizae were planted into various height PVC pipes in the greenhouses behind NSRFC did not work. The trees were all mycorrhizal when they were planted into the systems, but when the systems were disassembled there were very few trees with mycorrhizae. This explains why there were no apparent differences in the performance of the seedlings inoculated with the various fungi (see December 1987 report). It also underlines the importance of the fungi's ability to grow into the soils used in tests like this or in field trials. Apparently, the growing medium used in the tubes (non-sterilized peat-moss/vermiculite mixed with 2 g/l 20/20/20 slow release osmocote) was not appropriate for the persistence of these fungi.

The shortest of these tubes had been brought into the lab in November. After the seedlings flushed, they were subjected to drought and their APR was measured until it dropped to ca 5% of that measured at field capacity. The plants were then re-watered and the recovery rate was measured. No effects of the various fungal treatments were detected, which with hind-sight, is not surprising. I have not presented the data. The roots which the trees had formed since

planting into the tubes were stained and examined for mycorrhizae. Very few mycorrhizae were detected, indicating that the mycorrhizae had not extended from the original plug onto the roots that had formed since June.

In July, 1988, (13 months after the systems had been assembled) the seedlings that had been left outdoors in the tubes over the winter were removed from the tubes and inspected. Only 5 spruce trees had survived the winter and none of these had any mycorrhizae. There was no correlation between survival and the height (water status) of the tube or the fungus with which the trees had been inoculated. Twenty pine trees had survived. Again, there was no correlation between survival and the height of the tube in which the trees had been growing. Four of the trees had some H. longicaudum on their roots but the hyphae of these had not extended very far from the original root plug. Two of the seedlings had isolate 302 on their roots. This fungus had not spread very far into the surrounding mix either. The other species of mycorrhizae that had been put onto the roots were not detected.

In summary, the tube system did not show us very much. The tubes did not successfully impose a range of drought stresses on the seedlings as had been hoped. The taller tubes both became dry at about the same time, while the shorter ones remained wet. The fungi did not survive in the mix that we used, so very little can be said about their effects on tree performance under drought stress. It might be

worthwhile to re-do this trial using a soil that supported the introduced fungi and using more replicates per treatment. The latter could be facilitated by using only two tube sizes (short/wet and tall/dry).

The tube system did show that at least sometimes isolate 302 (2 seedlings) and H. longicaudum (5 seedlings) can survive outdoors under Nova Scotian conditions, since these fungi were both observed to be present when the systems were disassembled. Over-winter survival might be a critical factor differentiating fungi that would be good for use as inocula in Canadian forestry from those that would not. It is interesting to note that both isolate 302 and H. longicaudum are local isolates.